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Losartan-Dependent Regression of Myocardial Fibrosis Is Associated With Reduction of Left Ventricular Chamber Stiffness in Hypertensive Patients

Javier Díez, MD, PhD; Ramón Querejeta, MD, PhD; Begoña López, BSc; Arantxa González, BSc; Mariano Larman, MD; José L. Martínez Ubago, MD

Background—This study was designed to investigate whether myocardial collagen content is related to myocardial stiffness in patients with essential hypertension.

Methods and Results—The study was performed in 34 patients with hypertensive heart disease. Nineteen of these patients were also evaluated after 12 months of treatment with losartan. Transvenous endomyocardial biopsies of the interventricular septum were performed to quantify collagen volume fraction (CVF). Left ventricular (LV) chamber stiffness (K_{LV}) was determined from the deceleration time of the early mitral filling wave as measured by Doppler echocardiography. Histological analysis at baseline revealed the presence of 2 subgroups of patients: 8 with severe fibrosis and 26 with nonsevere fibrosis. Values of CVF and K_{LV} were significantly higher in the 2 subgroups of hypertensives than in normotensives. In addition, compared with patients with nonsevere fibrosis, patients with severe fibrosis exhibited significantly increased values of CVF and K_{LV}. After treatment, CVF and K_{LV} decreased significantly in patients with severe fibrosis (n=7). None of these parameters changed significantly after treatment in patients with nonsevere fibrosis (n=12). CVF was directly correlated with K_{LV} ($r=0.415$, $P<0.02$) in all hypertensives.

Conclusions—These findings show a strong association between myocardial collagen content and LV chamber stiffness in patients with essential hypertension. Our results also suggest that the ability of losartan to induce regression of severe myocardial fibrosis is associated with diminution of myocardial stiffness in hypertensive patients. **(*Circulation*. 2002; 105:2512-2517.)**

Key Words: collagen ■ hypertension ■ myocardium ■ peptides

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Myocardial fibrosis as a result of an exaggerated accumulation of collagen types I and III has been reported in the myocardium of patients with hypertensive heart disease (see Díez et al[1] for review). It has been proposed that the excess of myocardial collagen seen in hypertension is the result of both increased collagen synthesis and decreased collagen degradation.[2] Recent experimental[3,4] and clinical[5] findings suggest that the interaction of angiotensin II with its type 1 (AT_1) receptors plays a critical role in alterations of collagen type I metabolism and development of myocardial fibrosis in arterial hypertension.

Hypertension has been found to be associated with increased diastolic stiffness of the left ventricle.[6] It has been proposed that myocardial fibrosis is responsible for an increase in intrinsic myocardial stiffness that may alter left ventricular (LV) diastolic properties and the pattern of LV filling in the hypertensive heart.[7] LV stiffness is difficult to measure in clinical practice, even with invasive techniques. Thus, a number of noninvasive Doppler indexes have been reported that reflect increased LV stiffness, one of the most useful being the deceleration time of the early mitral filling wave (T_{DEC}). Shortened T_{DEC} has been associated with increased LV filling pressure in patients with different types of cardiomyopathies.[8–10] Recent studies in animals[11,12] and humans[13] with several different hemodynamic conditions have validated an analytical expression that is useful to calculate LV chamber stiffness (K_{LV}) from the measurement of T_{DEC}.

We thus hypothesized that in arterial hypertension, an association should exist between altered collagen type I metabolism, myocardial fibrosis, and increased LV stiffness. To test this hypothesis, the present study was designed to compare K_{LV} with histomorphometric assessment of myocardial collagen content (collagen volume fraction, CVF) and serum concentrations of carboxy-terminal propeptide of procollagen type I (PIP) and carboxy-terminal telopeptide of collagen type I (CITP), markers of collagen type I synthesis

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From the Division of Cardiovascular Pathophysiology (J.D., B.L., A.G.), School of Medicine, and the Department of Cardiology and Cardiovascular Surgery (J.D.), University Clinic, University of Navarra, Pamplona; the Division of Cardiology (R.Q.), Donostia Hospital, San Sebastián; and the Division of Hemodynamics (M.L., J.L.M.U.), Guipuzcoa Policlinics, San Sebastián, Spain.

Correspondence to Javier Díez, MD, PhD, División de Fisiopatología Cardiovascular, Facultad de Medicina, C/Irunlarrea 1, 31080 Pamplona, Spain. E-mail jadimar@unav.es

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and degradation, respectively,[14] in patients with essential hypertension. In addition, because chronic treatment with the AT_1 receptor antagonist losartan is associated with reduction of collagen type I synthesis and regression of myocardial fibrosis in hypertensives,[5] we also hypothesized that this compound should diminish K_{LV} in these patients. To test this hypothesis, the effects of chronic administration of losartan on PIP, CITP, CVF, and K_{LV} were investigated in hypertensives.

Methods

Patients and Study Protocol

All subjects gave written informed consent to participate in the study, and the local committee on human research approved the study protocol. The study conformed to the principles of the Declaration of Helsinki.[15]

The study population consisted of 34 white patients (22 men and 12 women; mean age 58 years; range 39 to 75 years) with systolic blood pressure and diastolic blood pressure of >139 and >89 mm Hg, respectively. All patients had appropriate clinical and laboratory evaluations to exclude secondary hypertension. Coronary artery disease was excluded as previously reported.[14]

Nineteen patients were treated for 1 year with losartan. No washout phase was performed so as to ensure continuous antihypertensive treatment (required by the ethics committee). Use of diuretic and antiadrenergic medications was reported by 2 and 7 patients, respectively. The dosage of losartan was titrated to achieve the therapeutic goal of systolic blood pressure and diastolic blood pressure <140 and <90 mm Hg, respectively. After titration, all patients were receiving a daily dosage of 50 mg.

A group of 10 hearts (from 6 men and 4 women; mean age 59 years; range 40 to 68 years) collected from a total of 100 autopsies performed at the University Clinic of Navarra during 1998 and 1999 served as controls for myocardial fibrosis after cardiac disease had been excluded. A group of 27 healthy subjects (18 men and 9 women; mean age 57 years; range 34 to 69 years) was also studied to establish the reference values for T_{DEC}, K_{LV}, PIP, and CITP.

Assessment of LV Mass, Function, and Stiffness

2D, targeted M-mode and Doppler ultrasound recordings were obtained in each patient as previously described.[14] LV mass and interventricular septal thickness were measured, and LV mass index was calculated by dividing LV mass by body surface area. The following pulsed-Doppler measurements were obtained: maximal early transmitral velocity in diastole ($\dot{V}_E$), maximal late transmitral velocity in diastole (V_A), isovolumic relaxation time, and T_{DEC}. Consistent with clinical practice, T_{DEC} was calculated as the time from the peak of the E wave to the zero-velocity intercept of the regression line of the E-wave velocity deceleration profile.[16] The ejection fraction was calculated from the measurements performed in a ^{99m}Tc ventriculography (multigated acquisition scan).

All patients exhibited hypertensive heart disease, as indicated by the presence of LV hypertrophy (defined as LV mass index >111 g/m^2 in men and >106 g/m^2 in women)[17] and/or diastolic dysfunction (defined as alterations in the $\dot{V}_E$-to-V_A ratio and/or isovolumic relaxation time and/or T_{DEC} according to García et al).[18] None of the patients studied exhibited systolic dysfunction, as assessed by an ejection fraction <50%.

K_{LV} was calculated as the ratio squared according to the following equation[11,13]: $K_{LV}=(0.07 : T_{DEC})^2$ mm Hg/mL.

Histomorphological Study

Transvenous endomyocardial biopsies were taken from the middle area of the interventricular septum with a bioptome (Cordis), 96 cm (7F), under fluoroscopic guidance after angiographic examination, as previously reported.[5,14] The rationale for the use of this procedure is based on previous findings that fibrosis present in the septum in postmortem tissue from hypertensive human hearts is representative of fibrosis existing in the free wall.[19] The biopsy procedure was well tolerated in all cases. CVF was determined by quantitative morphometry with an automated image analysis system (Visilog 4.1.5., Noesis) in sections stained with collagen-specific picrosirius red (Sirius red F3BA in aqueous picric acid) as previously described.[5,14]

Biochemical Determinations

Serum PIP was determined by radioimmunoassay according to a method previously described.[20] The interassay and intra-assay variations for determining PIP were 7% and 3%, respectively. The sensitivity (lower detection limit) was 1.20 μg PIP/L. Serum CITP was determined by radioimmunoassay according to Laviades et al.[21] The interassay and intra-assay variations for determining CITP were <8%. The sensitivity was 0.50 μg CITP/L. The PIP-to-CITP ratio was used as an index of coupling between the synthesis and degradation of collagen type I.[22]

Statistical Analysis

A cluster analysis was performed to classify hypertensive patients according to CVF values. Kruskal-Wallis 1-way ANOVA followed by a Mann-Whitney *U* test (adjusting the α-level by Bonferroni inequality) was used to assess the statistical significance between normotensives and the 2 subgroups of hypertensives. Baseline differences between the 2 subgroups of hypertensives were tested by a Student's *t* test for unpaired data once normality was demonstrated (Shapiro-Wilks test); otherwise, a nonparametric test (Mann-Whitney *U* test) was used. Differences between hypertensives before and after treatment were tested by a Student's *t* test for paired data once normality was demonstrated (Shapiro-Wilks test); otherwise, a nonparametric test (Wilcoxon test) was used. The correlation between continuously distributed variables was tested by univariate regression analysis. Values are expressed as mean±SD. A value of $P<0.05$ was considered statistically significant.

Results

Baseline Characteristics

Cluster analysis of CVF values allowed us to classify hypertensives into 2 subgroups; those with CVF <6% (n=26) and those with CVF >6% (n=8). Histological examination revealed that patients with CVF values <6% exhibited slight or moderate interstitial deposition of red-stained fibers. In contrast, patients with CVF values >6% presented areas of microscopic scarring and perivascular fibrosis. Thus, according to our previous classification of myocardial fibrosis in hypertension,[14] we classified patients with CVF <6% as

TABLE 1. Clinical Parameters Determined in Hypertensive Patients

Parameter	Nonsevere Fibrosis	Severe Fibrosis
Age, y	57±9	62±9
Sex, M/F	19/7	3/5
Time of hypertension, y	7.31±6.58	8.79±8.24
SBP, mm Hg	172±37	184±29
DBP, mm Hg	96±12	92±9
LVMI, g/m^2	129±42	126±30
IVST, mm	11.48±2.87	11.24±1.69
IVRT, ms	110.04±16.42	107.25±10.85
V_E:V_A	0.91±0.22	0.82±0.35
EF, %	60±8	65±6

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; LVMI, LV mass index; IVST, interventricular septal thickness; IVRT, isovolumic relaxation time; and EF, ejection fraction. Values are expressed as mean±SD or number of subjects.

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TABLE 2. Histological and Biochemical Parameters Determined in Normotensive Subjects and Hypertensive Patients

Parameter	Normotensives	Hypertensives With Nonsevere Fibrosis	Hypertensives With Severe Fibrosis
CVF, %	1.95±1.00	4.60±1.11*	7.93±0.97*†
PIP, μg/L	70.46±24.27	110.73±24.68*	156.88±33.89*†
CITP, μg/L	2.18±0.49	3.78±1.92	2.89±0.82
PIP:CITP	34.26±15.83	33.83±12.91	58.99±22.92*†

Values are expressed as mean±SD.

*$P<0.01$ vs normotensive subjects; †$P<0.01$ vs hypertensives with nonsevere fibrosis.

hypertensives with nonsevere fibrosis and patients with CVF >6% as hypertensives with severe fibrosis.

No significant differences were observed between the 2 subgroups of hypertensive patients in the different clinical and echocardiographic parameters tested at baseline (Table 1). Whereas LV hypertrophy was present in 18 patients (69%) with nonsevere fibrosis, it was diagnosed in 8 patients (100%) with severe fibrosis. The patterns of diastolic function were distributed in the 2 groups as follows: normal function in 2 patients (8%) with nonsevere fibrosis, delayed relaxation in 20 patients (77%) with nonsevere fibrosis and 2 patients (25%) with severe fibrosis, pseudonormal filling in 4 patients (15%) with nonsevere fibrosis and 3 patients (37.5%) with severe fibrosis, and restrictive filling in 3 patients (37.5%) with severe fibrosis.

CVF, PIP, and the PIP-to-CITP ratio were higher ($P<0.01$) in the 2 subgroups of hypertensives than in the group of normotensives (Table 2). In addition, CVF, PIP, and the PIP-to-CITP ratio were higher ($P<0.01$) in hypertensives with severe fibrosis than in hypertensives with nonsevere fibrosis (Table 2).

As shown in Figure 1, T_{DEC} was shorter in hypertensives with severe fibrosis (167±53 ms) than in hypertensives with nonsevere fibrosis (227±44 ms, $P<0.05$) and normotensives (242±19 ms, $P<0.001$). Thus, K_{LV} was increased in hypertensives with severe fibrosis (0.218±0.020 mm Hg/mL) compared with hypertensives with nonsevere fibrosis

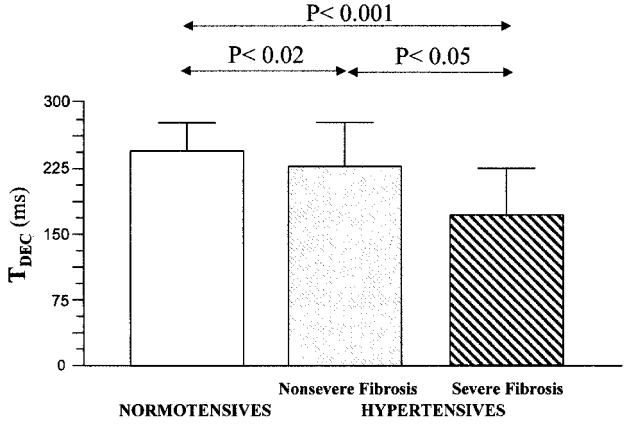

Figure 1. T_{DEC} in normotensive subjects and 2 subgroups of hypertensives classified according to grade of myocardial fibrosis. Bars represent mean+SD.

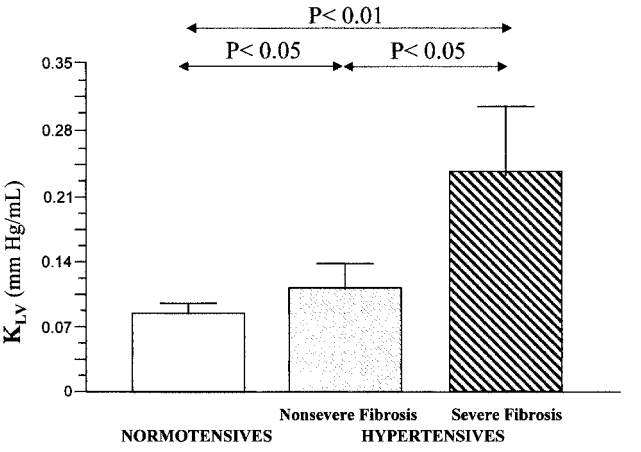

Figure 2. K_{LV} in normotensive subjects and 2 subgroups of hypertensives classified according to grade of myocardial fibrosis. Bars represent mean+SD.

(0.106±0.040 mm Hg/mL, $P<0.05$) and normotensives (0.085±0.014 mm Hg/mL, $P<0.01$) (Figure 2). In addition, T_{DEC} was shorter ($P<0.02$) and K_{LV} higher ($P<0.05$) in hypertensives with nonsevere fibrosis than in normotensives (Figures 1 and 2, respectively).

CVF was inversely correlated with T_{DEC} ($y=278.543-12.009x$, $r=0.415$, $P<0.02$) and directly correlated with K_{LV} ($y=0.043+0.024x$, $r=0.495$, $P<0.01$) (Figure 3) in all hypertensives.

Effects of Treatment

The effects of treatment with losartan were analyzed separately according to the baseline grade of fibrosis of treated patients. Therefore, the effects of treatment on hypertensives with nonsevere (n=12) and severe (n=7) myocardial fibrosis are presented in Tables 3 and 4, respectively.

Time-course changes in blood pressure during treatment (data not shown) and final values of blood pressure were similar in the 2 subgroups of hypertensives. Although LV mass index was diminished ($P<0.05$) after treatment in the 2

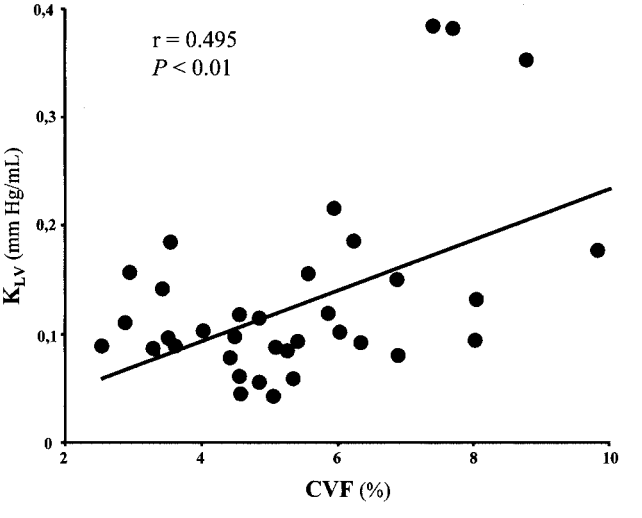

Figure 3. Direct correlation ($y=0.043+0.024x$) between CVF and K_{LV} in all hypertensive patients.

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TABLE 3. Effects of Losartan in Hypertensive Patients With Nonsevere Fibrosis

Parameter	Baseline	After Treatment
SBP, mm Hg	166±29	136±9†
DBP, mm Hg	97±12	82±11†
LVMI, g/m^2	130±30	110±21*
IVST, mm	11.58±1.92	10.66±1.92
IVRT, ms	112.33±19.10	103.67±15.97
V_E:V_A	0.97±0.28	0.96±0.09
T_{DEC}, ms	226.67±53.01	214.67±28.51
K_{LV}, mm Hg/mL	0.110±0.050	0.111±0.020
EF, %	60±7	59±6
CVF, %	4.32±0.98	3.72±1.50
PIP, μg/L	117.83±25.54	91.17±28.07
CITP, μg/L	3.26±1.68	3.84±1.95
PIP:CITP	39.23±11.77	28.85±12.55

Abbreviations as in Table 1. Values are expressed as mean±SD.
*$P<0.05$; †$P<0.01$.

subgroups of patients, interventricular septal thickness diminished ($P<0.05$) only in hypertensives with severe fibrosis. Isovolumic relaxation time, $\dot{V}_E$-to-V_A ratio, and ejection fraction did not change significantly with treatment in the 2 subgroups of patients. Treatment with losartan was associated with an increase ($P<0.05$) of T_{DEC} and diminution ($P<0.05$) of K_{LV} in hypertensives with severe fibrosis. The changes observed with treatment in these 2 parameters in hypertensives with nonsevere fibrosis did not reach statistical significance.

Whereas CVF diminished ($P<0.05$) with treatment in patients with severe fibrosis, a nonsignificant decrease in this parameter was observed in treated patients with nonsevere fibrosis. No correlation was found between the baseline and the posttreatment values of CVF either in the whole group of patients or in each subgroup. Thus, changes in CVF observed in patients with severe fibrosis are a result of the treatment per se. Figure 4 illustrates treatment-induced changes in myocardial fibrosis in 1 patient from each subgroup.

TABLE 4. Effects of Losartan in Hypertensive Patients With Severe Fibrosis

Parameter	Baseline	After Treatment
SBP, mm Hg	184±31	139±7*
DBP, mm Hg	90±9	84±9*
LVMI, g/m^2	129±30	97±19*
IVST, mm	10.83±1.33	9.27±0.99*
IVRT, ms	109.43±9.64	106.71±10.37
V_E:V_A	0.84±0.37	0.70±0.17
T_{DEC}, ms	162.43±51.31	220.71±48.88*
K_{LV}, mm Hg/mL	0.236±0.013	0.118±0.070*
EF, %	64±7	65±5
CVF, %	7.92±1.05	4.36±0.32*
PIP, μg/L	151.43±25.54	105.00±22.22*
CITP, μg/L	3.03±0.77	3.11±0.38
PIP:CITP	52.69±15.54	34.24±8.30*

Abbreviations as in Table 1. Values are expressed as mean±SD.
*$P<0.05$.

Losartan treatment was associated with a decrease ($P<0.05$) in both PIP and the PIP-to-CITP ratio in patients with severe fibrosis. Although the values of these 2 parameters did tend to decrease with treatment in patients with nonsevere fibrosis, the differences did not reach statistical significance.

Discussion

The main findings of this study are as follows: (1) a strong association exists between predominance of collagen type I synthesis over collagen type I degradation, exaggerated collagen accumulation in myocardial tissue, and abnormally high K_{LV} in patients with essential hypertension; (2) chronic AT_1 blockade with losartan is associated with reduction of both myocardial collagen content and K_{LV} in a subgroup of patients with essential hypertension; and (3) the efficacy of losartan in improving collagen type I metabolism predicts its capacity to regress myocardial fibrosis and reduce myocardial stiffness in hypertensive patients.

LV stiffness is difficult to measure even with invasive techniques, requiring high-fidelity pressure measurements and synchronized volume assessment with high temporal resolution. Thus, several noninvasive Doppler echocardiographic indexes of LV filling have been proposed as qualitative estimates of LV stiffness, including T_{DEC}. A shortened T_{DEC} has been associated with reduced ventricular compliance in patients with restrictive cardiomyopathies.[8–10] The lack of quantitative rigor in relating T_{DEC} to compliance, however, has limited the usefulness of this index. This has been resolved by experiments in an animal model of dilated cardiomyopathy that validated an analytical expression allowing us to calculate a value for LV stiffness, K_{LV}, from the measurement of T_{DEC}.[11,12] More recent studies have shown that a similar equation relating K_{LV} quantitatively to T_{DEC} applies to patients with cardiac diseases.[13]

By using this noninvasive approach, we observed that LV stiffness is abnormally increased in patients with essential hypertension. This finding is in agreement with data by Jain et al[6] showing that K_{LV} measured invasively from the diastolic pressure-volume relation is significantly higher in hypertensive patients than in normotensive control subjects.

LV stiffness is governed by a complex interplay of myocardial stiffness (largely related to the tissue collagen content),[23] ventricular geometry (hypertrophy[24] and distension[25]), and myocardial relaxation.[26] Our findings that K_{LV} in hypertensive patients is related to CVF but not to the degree of LV hypertrophy and relaxation abnormalities and that LV volumes were not increased in these patients (data not shown) strongly suggest that myocardial fibrosis is the main determinant of LV stiffness in essential hypertension. This is in accordance with data obtained in rats with spontaneous hypertension showing that myocardial fibrosis determines exaggerated LV stiffness.[27] Furthermore, our data provide clinical support to the proposal by Weber[7] that in hypertensive heart disease, a 2- to 3-fold or greater rise in CVF adversely influences LV diastolic stiffness.

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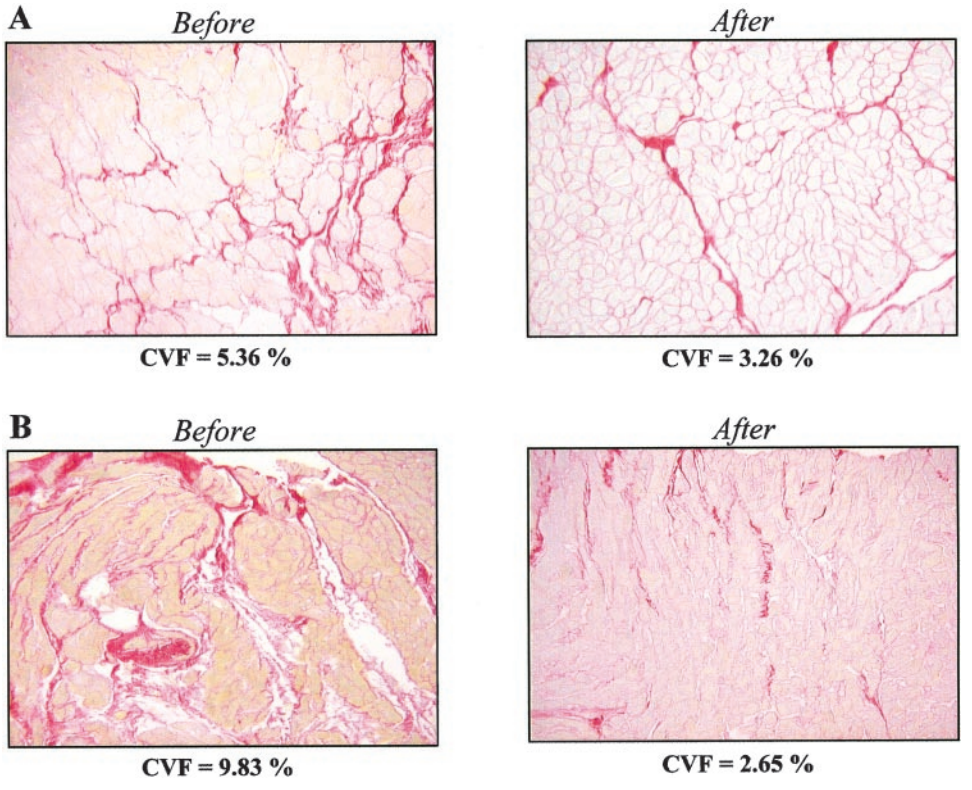

Figure 4. Histological section of myocardial specimen biopsy from a hypertensive patient with nonsevere myocardial fibrosis (A) and a hypertensive patient with severe myocardial fibrosis (B) before and after treatment with losartan. Picrosirius red stain; magnification ×20.

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Treatment with losartan was accompanied by a significant reduction in both CVF and K_{LV} in the subgroup of patients with severe fibrosis. In contrast, no significant changes in either CVF or K_{LV} were observed with treatment in hypertensives with nonsevere fibrosis. Brilla et al[28] reported recently that lisinopril-mediated diminution of 0.6% in CVF in patients with hypertensive heart disease was not associated with reduction in K_{LV}, as measured invasively. Thus, the ability of antihypertensive drugs to improve LV stiffness depends on their capacity to regress myocardial fibrosis in a significant manner.

Although we did not compare the response to losartan with that of another antihypertensive agent, our findings point out the potential relevance of angiotensin II as a factor that may compromise diastolic function in hypertensives via stimulation of myocardial fibrosis. This is supported by experimental data in rats with spontaneous hypertension showing that chronic ACE inhibition is associated with both regression of fibrosis and diminution of LV stiffness.[29,30]

Interestingly, whereas similar reductions in blood pressure and LV mass were attained in the 2 subgroups of hypertensives after treatment, only patients with severe fibrosis exhibited a significant reduction in the PIP-to-CITP ratio. Thus, the ability of losartan to improve LV diastolic properties in hypertensive patients appears to be linked to its capacity to repair myocardial fibrosis as the result of normalization of collagen type I metabolism.

In fact, if an equilibrium is to exist between collagen synthesis and degradation, as proposed by Laurent,[31] our findings of increased serum PIP concentrations and normal serum CITP concentrations in hypertensive patients suggest that the intensity of the extracellular degradation of collagen type I is not enough to equilibrate the increased extracellular synthesis of collagen type I, especially in those with severe fibrosis. The effect of losartan on serum PIP suggests that this agent inhibits the synthesis of collagen type I in hypertensives with severe fibrosis but not in hypertensives with nonsevere fibrosis. Conversely, the tendency toward increased CITP despite the decrease in PIP observed in treated hypertensives with severe fibrosis suggests that losartan stimulates the degradation of collagen type I fibers in these patients. Collectively, these observations suggest a direct role for angiotensin II–mediated collagen type I accumulation in the subgroup of hypertensives with severe myocardial fibrosis.

In summary, the results presented here show for the first time that in humans with hypertensive heart disease, exaggerated myocardial collagen content is associated with excessive K_{LV}, as assessed noninvasively. Thus, myocardial fibrosis may play a critical role in the compromise of diastolic function in patients with essential hypertension. This is further supported by our finding that alterations of diastolic function were more prominent in patients with severe fibrosis than in patients with nonsevere fibrosis. Nevertheless, we are aware that other criteria in addition to those of the present study should be used to adequately assess diastolic function in humans.[18] The potential epidemiological and clinical relevance of the findings presented here is provided by data showing that diastolic heart failure accounts for 30% to 50%

of congestive heart failure in clinical practice and that hypertensive heart disease is the major cause of this type of heart failure.[32]

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